Newcastle University ePrints

Schellekens J, Buurman P, Kuyper TW, Abbott GD, Pontevedra-Pombal X, Martínez-Cortizas A. Influence of source vegetation and redox conditions on lignin-based decomposition proxies in graminoid-dominated ombrotrophic peat (Penido Vello, NW Spain).
***Geoderma* 2015, 237-238, 270-282.**

Influence of source vegetation and redox conditions on lignin-based decomposition proxies in graminoid-dominated ombrotrophic peat (Penido Vello, NW Spain)

Judith Schellekens[ab*], Peter Buurman[c], Thomas W. Kuyper[d], Geoffrey D. Abbott[e], Xabier Pontevedra-Pombal[a], Antonio Martínez-Cortizas[a]

[a]*Departamento de Edafoloxía e Química Agrícola, Universidade de Santiago de Compostela, Fac. de Bioloxía- Campus Universitario Sur 15782 Santiago de Compostela (La Coruña), Spain*

[b]*Department of Soil Science, Escola Superior de Agricultura "Luiz de Queiroz", University of São Paulo, caixa postal 9, CEP 13418-900 Piracicaba (SP), Brazil*

[c]*Earth System Science Group, Wageningen University, P.O. Box 47, 6700 AA Wageningen, The Netherlands*

[d]*Department of Soil Quality, Wageningen University, P.O. Box 47, 6700 AA Wageningen, The Netherlands*

[e]*School of Civil Engineering and Geosciences, Drummond Building, Newcastle University, Newcastle upon Tyne NE1 7RU, UK*

[*]Corresponding author. Tel.: +31 653832087.

E-mail address: schellekens.j@hetnet.nl (Judith Schellekens).

Keywords

Peatland; Pyrolysis-GC-MS; Lignin degradation; C/N ratio; Graminoids; Ericoids.

1. Introduction

Ombrotrophic peatlands receive water and nutrients from precipitation alone. Although autogenic processes may influence the water table (Malmer et al., 1994), the depth of the water table within a given peatland is to a large extent dependent on precipitation. Water table depth determines oxygen availability and thereby it controls peat decomposition (Abbott et al., 2013; Philben et al., 2013). The degree of decomposition is therefore often used either as a proxy for climate (Blackford and Chambers, 1993), indicating the relative importance of drier and wetter periods, or to obtain information on the rate of organic carbon sequestration (Clymo et al., 1998).

Lignin is a major component of plant remains in peatlands, due to slow decomposition rates under anoxic conditions (Kirk and Farrel, 1987; Williams and Yavitt, 2003). Lignin is a macromolecule composed of syringyl (S), guaiacyl (G) and *p*-hydroxyphenyl (H) sub-units that are irregularly bound to each other. Its composition differs between major plant groups. In addition, non-woody tissue possesses, besides lignin, a high content of free and bound phenols that are ester- and/or ether-linked to lignin and polysaccharides in the cell wall (Hedges and Mann, 1979; Kondo et al., 1989; Lam et al., 1992; Sun et al., 2011). Apart from these source characteristics, the composition of lignin moieties can be influenced by side chain oxidation and shortening during decay (e.g. Kögel-Knabner, 2002). Thus, the composition of lignin in peat contains information about plant source and degradation state that may provide useful information about past environmental conditions (Kuder and Kruge, 1998; Bourdon et al., 2000; Zaccone et al., 2008; Disnar et al., 2008).

Knowledge of lignin transformation in soils and sediments is based mainly on aerobic decay (Thevenot et al., 2010) and woody tissue (Donaldson, 2001), while lignin research in graminoids has concentrated on forage quality (Buranov and Mazza, 2008). The effects of decomposition on graminoid tissue in peat have been studied by few authors (Kuder et al., 1998). Although anaerobic degradation of lignin proceeds at a slower rate (Opsahl and Benner, 1995) and lignocellulose can be completely degraded under anaerobic conditions (Benner et al., 1984), information about the pathways of anaerobic lignin degradation is scarce (Young and Frazer, 1987; van der Heijden and Boon, 1994). The effect of decay on the original lignin plant signal in environmental archives has been reviewed recently by Jex et al. (2014), who conclude that the interpretation of lignin proxy records demands a thorough understanding of the many processes that may be involved. In contrast, it has also been found that plant material affects the interpretation of lignin decomposition proxies in soils (Nierop and Filley, 2007; Mason et al., 2009). Because lignin composition varies between plant parts and elements of plant cells (Grabber et al., 2004), its resistance to decay may show a similar variation (van der Heijden et al., 1994; Williams and Yavitt, 2003; Machinet et al., 2011). Thus, in peatlands, with prevailing anaerobic conditions and significant contribution from graminoids, interpretation of lignin characteristics is intricate. Environmental interpretation of proxy records in peatlands is further complicated by the fact that changes in hydrology drive changes in both plant species composition and the nature and degree of decomposition (Yeloff and Mauquoy, 2006).

Pyrolysis-gas chromatography-mass spectrometry (pyrolysis-GC-MS) is applied frequently to study lignocellulosic material (Ralph and Hatfield, 1991; Meier et al., 1992). In addition, pyrolysis-GC-MS provides detailed information on the overall molecular composition, which benefits the interpretation of complex mixtures of

organic matter in peatlands and soils. The Penido Vello peat (Galicia, NW Spain) is dominated by graminoids, with significant contributions from ericoids at some depths. A high-resolution sampled peat core in this bog has been previously studied with pyrolysis-GC-MS. Schellekens et al. (2011) combined depth records of biomarkers of peatland plants with present-day plant ecology to reconstruct bog hydrology. Assigned wet and dry periods agreed well with other European studies. Thus, the Penido Vello bog, with its dominance of graminoids and known past vegetation shifts, is particularly suited to study the effects of decomposition on the lignin composition in peat.

To separate the effects of both source material and decomposition processes, a selection of fifteen samples from the same core were previously studied in detail by Schellekens et al. (2012). Analysis of peatland plants and comparison of NaOH-extractable organic matter (reflecting decomposition) and the non-extractable residue (reflecting source vegetation; Buurman et al., 2006) allowed separation of source effects (graminoid vs. ericoid material) and different decay processes on the peat lignin composition. The results indicated that during aerobic decay at the bog surface non-lignin phenolics are rapidly lost, and alkyl side chain reduction occurred for G moieties only. Depth related lignin decay (long term anaerobic decay) caused oxidation and reduction of alkyl side chains. Sub-surface (secondary) aerobic decay during water table drawdowns caused fragmentation of lignin while lignified cellulose was less affected. The effects were sometimes contrasting, for example G moieties were preferentially degraded during aerobic decay at the surface while S moieties were preferentially degraded during sub-surface aerobic decay. The results by Schellekens et al. (2012) were based on pyrolysates from two peat fractions that were not quantified. Because analytical pyrolysis provides relative instead of absolute abundances and because

relatively few samples were used, it remains unclear to which extent the results can be generalised and applied to bulk samples.

In order to evaluate the net effect of environmental factors, the present study applied a number of lignin-based parameters, extracted from Schellekens et al. (2012) to pyrolysates of 51 bulk samples from the upper meter of the Penido Vello peat core. To support the pyrolysis results, C/N and ^{13}C CPMAS NMR data were used. The purpose was to (i) identify and separate the effects of botanical shifts and several decomposition stages (aerobic and anaerobic) on the lignin composition in bulk samples, and (ii) examine the use of lignin-based decomposition proxies as a tool for (palaeo)climatic interpretation.

2. Methods

2.1. Location and sampling

Penido Vello is an ombrotrophic mire in the Xistral Mountains (Galicia, NW Spain). Location, sampling and characteristics of the bog have been described in detail by Martínez-Cortizas et al. (1997, 2002). The 3 m deep core dates back to 6000 yr BC. For this study, only the samples from the upper 1 m were used (51 continuous samples of 2 cm in thickness), as this section showed better correlation between vegetation markers than the deeper part (Schellekens et al., 2011), which had been sectioned into 5 cm slices. It represents ca. 2000 yr of peat accumulation. Samples were dried at 35 °C (1 week) and ground for pyrolysis without further treatment.

2.2. Vegetation shifts and characteristics in the Penido Vello peat record

The ombrotrophic section of the peat core was dominated by the graminoids, *Carex durieui*, *Agrostis curtisii*, *Molinia caerulea*, with significant contributions from ericoids

(*Erica mackaiana* and *Calluna vulgaris*), *Eriophorum angustifolium*, *Festuca rubra* and mosses at some depths. Although *Sphagnum* was present in most samples its contribution to the peat biomass was low. Major vegetation shifts upon changes in hydrology have been found between *C. durieui*, *A. curtisii*, *F. rubra*, *E. mackaiana* and *C. vulgaris* (drier conditions) and *M. caerulea*, *E. angustifolium* and moss (wetter conditions; Fraga et al., 2005).

The contribution of roots to the chemistry of older peat layers may complicate a correct palaeohydrological interpretation. Plant morphology, growth characteristics, and good correlations between depth records of peatland plant biomarkers with those of decomposition proxies, indicate that such root effects are negligible for Penido Vello. This aspect is discussed in detail by Schellekens et al. (2011). It is assumed that a loss of lignin phenols as dissolved organic matter is negligible because the peat is located on the summit of a mountain, without any evidence of an outlet.

2.3. Selection of lignin parameters

Based on the results of the preliminary study by Schellekens et al. (2012), a number of lignin-based decomposition parameters were extracted. They are given in Table 1 and structure formulae of associated lignin pyrolysis products are given in Fig. 1.

2.4. Total carbon and nitrogen

The samples were analysed for total carbon and total nitrogen, as described by Pontevedra-Pombal et al. (2004).

2.5. Solid state ^{13}C CPMAS NMR spectroscopy

145 The Penido Vello core was previously analysed with ^{13}C CPMAS NMR, mean
146 values for each stratigraphic layer (Pontevedra-Pombal et al., 2001) and depth records
147 of the whole core (Pontevedra-Pombal et al., 2004) are already published. The ^{13}C
148 CPMAS NMR spectra were divided into chemical shift regions: 0–46 ppm, aliphatics;
149 46–60 ppm, methoxyl; 60–95 ppm, O-alkyl C; 95–110 ppm, acetyl; 110–140 ppm,
150 aromatics; 140–160 ppm, phenolics; and 160–250 ppm, carboxyl (Pontevedra-Pombal
151 et al., 2001; Kaal et al., 2007). For a detailed description of the methodology we refer to
152 these studies.
153
154 *2.6. Pyrolysis-GC-MS*
155 A Curie-Point pyrolyser (Curie temperature 600 ºC) connected to a Carlo Erba
156 GC8000 gas chromatograph was used. Pyrolysis products were separated on a non-polar
157 fused silica column [Chrompack: 25 m, 0.25 mm i.d., CP-Sil 51 b (0.40 µm)], with He
158 as carrier gas. The oven temperature programme was: 40 ºC to 320 ºC (held 15 min) at 7
159 ºC min^{-1}. The column was connected to a Fisons MD800 mass spectrometer (*m*/*z* 45–
160 650, cycle time 1 s).
161 Quantification in the present study was identical to that of Schellekens et al. (2012;
162 Appendix) and based on the peak area of two major fragment ions from each pyrolysis
163 product. All individual quantifications were checked manually. For each sample, the
164 sum of the peak areas was set at 100 % and proportions were calculated relative to this.
165 According to probable origin and similarity, the pyrolysis products were grouped as
166 follows: polysaccharides, aliphatic hydrocarbons, methyl ketones, lignin products,
167 phenols, catechols, (other) aromatic hydrocarbons, polyaromatic hydrocarbons, nitrogen
168 containing compounds, fatty acids, steroids and triterpenoids (Appendix).
169

170 *2.7. Statistical analysis*

171 In order to identify and separate the effects of the main environmental
172 factors/processes influencing peat lignin composition of the 51 analysed samples, factor
173 analysis was applied using 120 quantified pyrolysis products (Appendix; Schellekens et
174 al., 2012) and 15 lignin parameters (Table 1; Section 2.3). The identification of the
175 factors was done using the explained variance of the pyrolysis products as reflected by
176 the factor loadings. Thereafter, the contribution of the factors to the variation in lignin
177 parameters was determined. The factor scores were used as a measure of the
178 effect/weight of the factor on the composition of any given sample and allow
179 comparison with other characteristics such as ^{13}C NMR and C/N. Factor analysis was
180 performed with Statistica® Version 6 (Statsoft Inc, Tulsa). All correlation coefficients
181 had a significance level of $P < 0.00001$.

182

183 **3. Results and Discussion**

184 The identification of the effects of environmental factors on the lignin parameters is
185 described in Section 3.1; the net effect underlying the extracted factors is discussed
186 according to depth profiles of the lignin parameters in Section 3.2, and their
187 palaeohydrological value is discussed in Section 3.3.

188

189 *3.1. Factor analysis*

190 The first four extracted factors explained the major part of the total variance
191 (69.3%). Projections of the loadings of factors 1 and 2 (F1, F2) and factors 3 and 4 (F3,
192 F4) are given in Fig. 2. F1 (36.3% explained variance) is mainly characterised by
193 positive loadings of lignin phenols and short to mid-chain *n*-alkanes ($C_{10–25}$) and
194 negative loadings of polysaccharide pyrolysis products and long chain *n*-alkanes ($C_{29–33}$;

195 Fig. 2a). In peat, polysaccharides are preferentially degraded over lignin (Benner et al.,
196 1984; van der Heijden et al., 1990; Disnar et al., 2008) and an increase in aerobic decay
197 causes chain length reduction of *n*-alkanes (Eglinton and Hamilton, 1967; Schellekens
198 and Buurman, 2011). F1 is therefore assigned as reflecting bog hydrology at the time of
199 peat formation and thus the first stage of decay.

200 The high positive loading of aliphatic products on F2 (12.9% explained variance;
201 Fig. 2a) indicated residual accumulation of these compounds upon diagenesis, as an
202 increase in aliphatics was found for the whole (3 m) peat record with ^{13}C CPMAS NMR
203 (Pontevedra-Pombal et al., 2001; Buurman et al, 2006) and pyrolysis-GC-MS
204 (Schellekens et al., 2011). This indicates that F2 reflects long term anaerobic decay.

205 F3 (11.5% explained variance) showed high negative loadings for a sterol derived
206 (S3) and an aliphatic (Al2) pyrolysis product, that were obtained solely from ericoids
207 and woody plants, respectively (Schellekens et al., 2011), and long chain *n*-alkanes
208 (Schellekens and Buurman, 2011). Compounds with positive loadings on F3 were
209 associated with lignocellulose of graminoids: 4-vinylguaiacol from ferulic acid (Lg12)
210 and 4-hydroxy-5,6-dihydro-(2*H*)-pyran-2-one (Ps4; Section 3.2.4). Therefore, F3
211 separates ericoids (negative loadings) from graminoids (positive loadings, Fig. 2b).

212 F4 (8.6% explained variance) showed positive loadings for lignin products with a C_3
213 alkyl side chain that reflect unaltered lignin (Lg13, Lg16, Lg17, Lg31, Lg33, Lg37; van
214 der Hage, 1993; Kuder and Kruge, 1998), while benzene (Ar1), toluene (Ar2), pyridine
215 (N1) and polyaromatic products (PA1, PA2) showed negative loadings and are
216 associated with aerobic decay (Schellekens et al., 2009). F4 is therefore assigned as
217 reflecting secondary aerobic decay that occurs due to events of lowering of the water
218 table. Positive loadings on F4 indicate relatively undecomposed material (Fig. 2b) and
219 thus high water table during the development of the layer.

220 The first factor extracted showed significant correlation with data obtained from ^{13}C
221 CPMAS NMR (Fig. 3; Table 2): negative correlation of O-alkyl C (r^2 0.82) and acetal C
222 (r^2 0.66) was found with F1, while aromatic C (r^2 0.86) and phenolic C (r^2 0.77) were
223 positively correlated with F1, reflecting preferential decay of polysaccharides over
224 lignin. The agreement between pyrolysis and ^{13}C CPMAS NMR data indicates that the
225 trends obtained with analytical pyrolysis are valid despite the semi-quantitative
226 character of the method (see Section 2.6).
227 The identified factors (F1–F4) that influenced peat chemistry are summarised in
228 Table 3. During the first stage of decay at the surface, aerobic (water table below the
229 surface) and anaerobic conditions (water table at or above surface) altered the organic
230 matter differently. In the sub-surface, secondary aerobic decay may occur when the
231 water table lowers during drier phases. Although the degree of decay reflects a
232 continuum, primary and secondary aerobic decay showed different effects on peat
233 chemistry. Once in the anaerobic zone (catotelm), the peat is subject to long-term decay.
234 The variance of each individual lignin parameter accounted for by the four factors is
235 reflected by the fractionation of communalities (Fig. 4). Communalities for the
236 remaining factors (F5–F16) and individual pyrolysis products are not shown.
237
238 *3.2. Effects of vegetation composition and decay processes on the lignin parameters*
239 *3.2.1. Abundance of lignin and polysaccharides (parameter 1)*
240 The summed lignin (parameter 1a) and summed polysaccharide (parameter 1b)
241 pyrolysis products were for > 80% explained by F1 (Fig. 4), reflecting bog hydrology at
242 the time of peat formation (Table 3). High values of parameters 1a and 1b indicate
243 relatively dry and wet periods, respectively (Fig. 2), and reflect the preferential decay of
244 polysaccharides over lignin during the first stage of decay. The remaining variance of

polysaccharides (parameter 1b) was explained by F2 and F3 (Fig. 4) and is discussed in Section 3.2.4. The remaining variance in lignin (parameter 1a) was explained by F2 (Fig. 4) and is predominantly related to G moieties (Fig. 2a; Section 3.2.2.1).

3.2.2. Preferential decay of lignin moieties (parameter 2)

3.2.2.1. Syringyl to guaiacyl ratio

The analysis of different peat fractions indicated that differentiation between S and G moieties (parameter 2a) was predominantly related to decomposition instead of vegetation shifts (Schellekens et al., 2012). To exclude a contribution from non-lignin phenolic acids to the abundance of lignin moieties, only the ratio of S to G units with a C_3 alkyl side chain was chosen (parameter 2b), as these lignin moieties reflect macromolecular lignin (Kuroda, 2000; van der Hage et al., 1993). Demethoxylation during pyrolysis is negligible (Harman-Ware et al., 2013) and can be excluded.

Both S/G ratios, parameters 2a and 2b, were explained for > 85% by the first four factors, of which the major part was related to F1 (Fig. 4). The projection of parameters 2a and 2b was negative on both factors F1 and F2 (Fig. 2a), and their depth profiles showed an increase until 80 cm from where they stabilised (Fig. 5). This pattern indicated preferential decay of G moieties, both during primary decay at the surface (F1) and during long term anaerobic decay (F2). For parameter 2a an additional part of the variance was explained by F4, reflecting secondary aerobic decay in the sub-surface (Fig. 4). Parameter 2a de-trended for F1 and F2 (i.e. eliminating the effects of F1 and F2) showed a good positive correlation with F4 (r^2 0.63, not shown), indicating that, during secondary aerobic decay, S moieties were preferentially degraded over G moieties. The very low explained variance on F4 for parameter 2b (Fig. 4) indicates that

preferential decay of S moieties is not valid for lignin C_3 alkyl side chains (see also Section 3.2.3).

The preferential decay of G moieties was attributed by Schellekens et al. (2012) to a high contribution from a non-lignin phenolic acid source and thus not from macromolecular lignin. An increase in S/G ratio under anaerobic conditions was also found for graminoid tissue by Opsahl and Benner (1995) and in graminoid-dominated peat by Kuder et al. (1998). Preferential decay of S lignin, related to its structure, is generally described from aerobic systems (Thevenot et al., 2010) except during the earliest stages of decay (Christman and Oglesby, 1971; Kögel, 1986). Because anaerobic conditions predominate in peat, this first stage of decay is prolonged, and the results thus correspond well with current knowledge. However, because moieties with a C_3 alkyl side chains originate from the lignin macromolecule and parameters 2a and 2b showed similar behaviour in factor analysis, an additional explanation may be that of differential decay of plant tissues. Because structure and chemical composition of plant material may differ greatly within both plant parts and plant cells (Buranov and Mazza, 2008), it is likely that decay is connected to the anatomical structure of the plant, and thus to the accessibility of S or G lignin. This is in agreement with Grabber et al. (1997, 2004) whose results indicate that the degradability of grass cell walls was not affected by the composition of lignin moieties.

The graminoid species that dominate the Penido Vello peat have similar morphology, with thin leaves and a rigid stem (Schellekens et al., 2011), which suggests that the leaves are more easily degradable than the stems. According to macrofossil analysis of a similar peat record located at 2 km distance (Pena da Cadela), the peat is dominated by roots, rhizomes and internodes of graminoids. Remains of epidermis (which may originate from both leaves and stems) are also abundant but are much

294 smaller in size and fragmented (I. Fraga, personal communication). The preferential
295 decay of leaves vs. other plant organs in the peat may explain (part of) the abundance of
296 G and S lignin during decay. This is supported by lignin studies of other graminoids. In
297 brome grass (Jung and Casler, 1990) and barley (Love et al., 1998), S lignin was found
298 in higher abundance in the stems than in the leaves and was more resistant to decay than
299 G lignin, while the latter had a much higher abundance in the middle lamellae and was
300 more easily decayed (Love et al., 1998). Furthermore, an increase in S/G with maturity
301 in the stem of several graminoids (Grabber et al., 2004) was negatively correlated with
302 enzymatic degradability in *Festuca arundinacea* (Chen et al., 2002). Thus, in addition
303 to preferential decay of G moieties from non-lignin phenolic acids, the observed
304 decrease in G lignin under both aerobic and anaerobic conditions during the first stage
305 of decay (Table 2) is presumably related to plant anatomy. In woody tissue (e.g. from
306 ericoids) the lignin composition and plant architecture is different and thus preferential
307 decay of S moieties in peat in which ericoids determine the lignin content (van der
308 Heijden and Boon, 1994; Huang et al., 1997) does not necessarily contradict to the
309 preferential decay of G moieties in graminoid peat.
310 The source material (graminoids) therefore exerts a strong influence over
311 'preferential decay' of G or S moieties in peat. In agreement with this conclusion is the
312 fact that F3, which differentiated graminoids from ericoids, showed negligible
313 explained variance for both S/G ratios (Fig. 4). Thus, for peat dominated by graminoids,
314 parameters 2a and 2b would not be related to *shifts* between graminoids and ericoids,
315 but would be almost completely determined by decay (F1, F2 and F4).
316
317 *3.2.2.2. p-Hydroxyphenyl moieties*

318 Parameter 2c reflects the relative abundance of H moieties within the lignin
319 macromolecule. Lignin moieties with a vinyl side chain (4-vinylphenol Lg1, 4-
320 vinylguaiacol Lg12 and 4-vinylsyringol Lg30) were excluded from this parameter
321 because of their dual origin from both non-lignin phenolic acids and macromolecular
322 lignin (Section 3.2.4).
323 The first four factors explained only 52% of parameter 2c (Fig. 4). F3, which
324 distinguished graminoids from ericoids, accounted for the largest part of the explained
325 variance. Thus, although demethylation during the first stage of decay and long term
326 anaerobic decay occurs in peat (Tsutuki, 1994; Filley 2003; Schellekens et al., 2012),
327 the influence of such decay (F1, F2) was lower than that of source vegetation. The
328 negative loadings on F3 (Fig. 2b) indicated that H moieties had a relatively higher
329 abundance in the peat samples with a higher contribution of ericoids. This higher
330 abundance in ericoids contradicts the general interpretation of H lignin as being
331 indicative for grass (Hedges and Mann, 1979; Thevenot et al., 2010), and is presumably
332 related to the fact that H moieties may also originate from non-lignin phenolic polymers
333 such as tannins (Wilson et al., 1985), that have a higher abundance in woody species
334 (Kögel-Knabner, 2002).
335
336 *3.2.3. Side chain oxidation and shortening (parameters 3 and 4)*
337 Side chain oxidation highly increases from peatland plants to peat OM (Schellekens
338 et al., 2012). A commonly used parameter for lignin degradation is the acid/aldehyde
339 ratio (Ac/Ad) of G and S moieties after oxidation with CuO (Ertel and Hedges, 1985;
340 Thevenot et al., 2010). With pyrolysis however, G and S moieties with a C_3 alkyl side
341 chain, parameters 4a and 4b respectively, are generally interpreted as indicating intact
342 lignin (Mulder et al., 1991; van der Heijden and Boon, 1994). For the present samples,

343 the Ac/Ad ratio was completely determined by the acid moieties (not shown).
344 Therefore, vanillic acid and syringic acid were expressed as percentage of G and S,
345 parameters 3a and 3b, respectively. In addition to the acids, 4-acetylguaiacol and 4-
346 acetylsyringol were chosen to reflect oxidation of lignin alkyl side chains (parameters
347 3c and 3d, respectively).
348 Although vanillic and syringic acid were expected to increase upon (secondary)
349 aerobic decay in peat, parameters 3a and 3b had no loadings on F4 and a major part of
350 the observed change was related to undecomposed organic matter (F1) and ericoids (F3;
351 Fig. 4). Low Ac/Ad values that did not correspond to other degradation parameters were
352 also found by Disnar et al. (2008) in a sedge-dominated peat. An explanation may be
353 that a high abundance of acids in graminoid-dominated systems is related to a change in
354 vegetation composition. The knowledge that vanillic and syringic acid result from
355 fungal oxidative cleavage of C_3 alkyl side chains is based on wood lignin (Saiz-Jimenez
356 and de Leeuw, 1984; Hedges et al., 1988; Higuchi, 1990). Parameter 3a showed a
357 negative correlation with F1 only for the samples that had negative scores on F1 (r^2
358 0.64, n = 22; not shown). For these samples, vanillic acid may have originated from
359 non-lignin phenolic monomers known to be abundant in vascular plants (Geissman and
360 Crout, 1969) and were found abundantly in peat dominated by graminoids (Kuder et al.,
361 1998; Schellekens et al., 2012). Furthermore, ester-bound vanillic acid is found in high
362 amounts in seagrasses (Opsahl and Benner, 1993). The negative loading on F1 is then
363 explained by the rapid degradation of non-lignin phenolic monomers (Section 3.2.4).
364 Both acetyl ratios, parameters 3c and 3d, were positively correlated (r^2 0.86, not
365 shown) and showed evidently higher values during a wet period that was assigned to the
366 Little Ice Age (Fig. 5; Schellekens et al., 2011). Both showed a similar fractionation of
367 the communality with negative loading on both F1 and F4 (Figs. 2, 4). Their similar

behaviour indicates that they increased under both relatively wet (negative on F1; Fig. 2a) and secondary dry conditions (negative on F4; Fig. 2b), which may explain the absence of spikes in their depth profiles (Fig. 5). The clearly higher values during the Little Ice Age are explained by the duration of prevailing wet conditions. The initial increase in parameters 3c and 3d (upper 14 cm) suggest that the acetyl side chain is a result of anaerobic decay and cannot be interpreted as preservation due to the absence of aerobic decay. Thus, the formation of acetyl side chains occurs when the first step of aerobic decay is negligible and fresh material is subjected first to anaerobic conditions.

For the S moieties, the abundance of C_3 syringols (parameter 4b) was negatively correlated with parameter 3d (r^2 0.74, not shown), which suggests that C_3 syringols were transformed to 4-acetylsyringols during anoxic conditions at the first stage of decay. Anaerobic conditions during the first stage of decay (F1) and secondary aerobic decay (F4) explain the major part of the variance of parameter 4b (Fig. 4). Depletion of lignin moieties with a C_3 alkyl side chain also correlated with an increase in 4-acetylsyringol and 4-acetylguaiacol in peat investigated by Kuder and Kruge (1998). Although the effect of F4 dominated (Fig. 4), the abundance of C_3 syringols was obviously lower in the peat sections corresponding to the Little Ice Age (Fig. 5). A low abundance of C_3 syringols under wet conditions is not in agreement with the literature (Kuroda, 2000; van der Hage et al., 1993), as high abundances of lignin moieties with C_3 alkyl side chains are commonly interpreted as being from relatively intact lignin and thus indicating wet conditions (Philben et al., 2013).

Unlike the S moieties, for G moieties no correlation was found between C_3 and acetyl- alkyl side chains (r^2 0.01 for parameters 4a and 3c). C_3 guaiacols were mainly explained by F4 (Fig. 4) and showed particularly high values in the upper two samples (Fig. 5), indicating rapid decay of C_3 guaiacols. These rapidly degraded C_3 guaiacols

393 probably originate from graminoids (Schellekens et al., 2012). Nevertheless, a clear
394 maximum for parameter 3c occured during the Little Ice Age. This negative correlation
395 between lignin moieties with C_3 alkyl and acetyl side chains, suggests that the former
396 are oxidised during anaerobic conditions. This interpretation is supported by the good
397 correlations of other lignin pyrolysis products with an oxidised side chain with
398 parameters 3c and 3d. These include a correlation of syringic acid methyl ester (Lg38)
399 with parameter 3d (r^2 0.84); vanillic acid methyl ester (Lg21) with parameter 3c (r^2
400 0.57); and 4-(propan-1-one)guaiacol (Lg23) with parameter 3c (r^2 0.55; not shown). It is
401 not clear which mechanisms underlie these results.
402 A possible explanation is that, during prevailing wet conditions, the undecomposed
403 material, still rich in easily degradable polysaccharides, is anaerobically degraded by
404 bacteria. During the first step in anaerobic cellulose degradation various simple O-
405 containing compounds are released (Béguin and Aubert, 1994), which might be used for
406 the oxidation of lignin alkyl side chains in non-enzymatic Fenton based reactions as has
407 been found for demethylation of wood lignin by brown rot fungi (Sinsabaugh et al.,
408 2010; Filley et al., 2002).
409
410 *3.2.4. Non-lignin phenolic acids, hemicellulose and cellulose (parameters 5-7)*
411 Bourdon et al. (2000) used the ratio of cinnamic acids (i.e. ferulic acid and *p*-
412 coumaric acid) to lignin as an indicator of Cyperaceae. Typical pyrolysis products of *p*-
413 coumaric and ferulic acid are 4-vinylphenol and 4-vinylguaiacol, respectively (Boon et
414 al., 1982; van der Hage et al., 1993). From analysis of peatland plants it appeared that 4-
415 vinylguaiacol, from ferulic acid, was particularly associated with graminoids (parameter
416 5b), while 4-vinylphenol from *p*-coumaric acid (parameter 5a) showed a higher
417 abundance in graminoids that perform better under wet conditions (Section 2.2). Also

ericoids showed a relatively high abundance of 4-vinylphenol (Schellekens et al., 2012). However, 4-vinylguaiacol and 4-vinylphenol also originate from intact lignin (Harman et al., 2013) and chain length reduction of C_3 alkyl side chains during decay, which occurred under both aerobic and anaerobic conditions (Schellekens et al., 2012). Therefore, the presence of phenolic acids cannot be evaluated properly via pyrolysis-GC-MS without methylation (del Rio et al., 2007) and the interpretation of 4-vinylguaiacol and 4-vinylphenol can be problematic.

Xylose, which is abundant in hemicellulose of grasses (Wende and Fry, 1996; Smith and Harris, 1999) and sedges (Bourdon et al., 2000), results in 4-hydroxy-5,6-dihydro-(2*H*)-pyran-2-one upon pyrolysis (parameter 6; Pouwels et al., 1987). The main pyrolysis product from cellulose is levoglucosan (Pouwels et al., 1989), of which non-lignified cellulose is reflected by parameter 1b and lignified cellulose by parameter 7.

The variance of parameter 5b is mostly explained by F3 (64%; Fig. 4) and is related to graminoids (Fig. 2b), while the explained variance of parameter 5a is more distributed among other factors. Ferulic acid and *p*-coumaric acid are rapidly degraded once the plant dies (Kuder and Kruge, 1998; Bourdon et al., 2000). A large part of both compounds is presumably already lost before it enters the peat. A smaller part is more resistant to decay (Tareq et al., 2006), which agrees with the maxima in parameters 5a and 5b during wet periods, except for a minimum during the Little Ice Age of parameter 5b (Fig. 5). These clearly lower values of 4-vinylguaiacol may indicate that ferulic acid was transformed to 4-acetylguaiacol under initial persistent wet conditions, as suggested in Section 3.2.3 for lignin C_3 alkyl side chains. Anaerobic degradation of ferulic acid can occur, with acetate being formed as an intermediate during metabolism (Healy et al., 1980), which supports this idea. Because the Penido Vello peat is dominated by graminoids and vegetation shifts upon lowering of the water table may be more

443 prominent within graminoids than between ericoids and graminoids, the positive
444 loadings of parameters 5a and 5b on F3 may also be related to graminoids that perform
445 better under relatively wet conditions (*Eriophorum* and *Molinia*), as these showed
446 particularly high values of non-lignin phenolic acids (Schellekens et al., 2012).
447 The most abundant polysaccharide pyrolysis product in the peat was levoglucosan
448 (Appendix). The loss of polysaccharides during primary decay originates mainly from
449 non-lignified cellulose, which is easier to access. Assuming that non-lignified cellulose
450 is degraded as rapidly as other plant polysaccharides, levoglucosan expressed as
451 percentage of the total polysaccharide pyrolysis products reflects lignified cellulose
452 (parameter 7). The increase with depth in parameter 7 (Fig. 5) showed the relative
453 accumulation of lignified cellulose in an anaerobic environment (van der Heijden and
454 Boon, 1994; Huang et al., 1997; Disnar et al., 2008; Fig. 4). The separation between
455 parameters 6 and 7 on F2 (Fig. 2a), indicates that, during long term anaerobic decay,
456 hemicellulose is preferentially degraded over lignified cellulose. The slightly positive
457 loadings of parameter 7 on F1 also indicates that levoglucosan is less depleted than
458 other polysaccharide pyrolysis products during primary decay (Fig. 2a), indicating that a
459 large part of the cellulose was lignified. The negative loading of parameter 7 on F4
460 shows that, during secondary aerobic decay, lignified cellulose is degraded.
461
462 *3.3. Palaeoclimatic implications*
463 The application of the parameters to reconstruct environmental conditions from peat
464 records or to predict the effects of climate change related to the carbon stored in
465 peatlands is intricate. The variation with depth of most of the parameters depended on
466 several factors, the contribution of the different decay processes and vegetation changes

to each of them is indicated in Fig. 4. Their usefulness as proxies for indicating bog hydrology is related to aerobic decay at the surface.

3.3.1. Lignin and polysaccharide content (parameter 1)

Both polysaccharide and lignin content indicate bog hydrology at the moment of peat formation, and reflect the preferential decay of polysaccharides over lignin. The lignin/polysaccharide ratio may thus be a reliable indicator of bog hydrology (Tsutsuki et al., 1994; Schellekens et al., 2011; Broder et al., 2012).

3.3.2. Syringyl, guaiacyl and p-hydroxyphenyl moieties (parameter 2)

In the Penido Vello core, the S/G ratio was completely determined by decomposition (Section 3.2.2.1). However, interpretation is complex and highly dependent on prevailing decay phases. The decrease in G lignin during the first stage of decay and long term anaerobic decay is presumably related to preferential decay of non-lignin phenolic acids (Schellekens et al., 2012); an additional explanation is differential decay of plant parts that contain higher G content (leaves). During secondary aerobic decay the more resistant plant parts and macromolecular lignin are accessible and S is preferentially lost.

Although parameter 2c had the lowest explained variance, the major part was related to source, namely ericoids. This confirms that a high abundance of H moieties in ericoids as obtained from peatland plant analysis (Schellekens et al., 2012) can still be related to its source in peat, notwithstanding the graminoid dominance in the peat core. The contrast with the general assumption that a high abundance of H moieties in soils is related to graminoids (Thevenot et al., 2010), indicates that parameter 2c is not always a trustworthy indication of source material.

492

493 *3.3.3. Alkyl side chain oxidation (parameter 3)*

494 Side chain oxidation is generally related to fungal degradation and therefore used to
495 indicate aerobic conditions in peat records. In the Penido Vello core, half of the
496 variation in vanillic and syringic acid was related to ericoids, and the other half to non-
497 lignin phenolic acids from graminoids preserved under anoxic conditions during the
498 first stage of decay (Fig. 4). Thus, a high abundance of vanillic and syringic acid may
499 indicate (i) relatively wet conditions during the first stage of decay or (ii) an ericoid
500 source, and thus indirectly relatively dry conditions. The absence of a relationship with
501 secondary aerobic decay may be due to the fact that, under anoxic conditions,
502 lignocellulose from graminoids is degraded more rapidly than that from wood,
503 suggesting that wood is not yet strongly degraded.

504 Similarly acetyl side chains were related to both relatively dry (secondary aerobic
505 decay) and wet (first stage of decay) conditions. The fact that the effect of anaerobic
506 decay was more prominent in the depth records (Fig. 5) may be due to the long duration
507 of the Little Ice Age.

508

509 *3.3.4. Alkyl side chain reduction (parameter 4)*

510 A large part of the C_3 alkyl side chains is degraded during secondary aerobic decay.
511 However, the interpretation of lignin moieties with a C_3 alkyl side chain to reflect intact
512 lignin may lead to erroneous hydrological interpretation, as C_3 syringols in particular
513 were depleted during wet periods. Neither of both parameters (4a and 4b) was correlated
514 with bog hydrology at the time of peat formation.

515

516 *3.3.5. Non-lignin phenolic acids (parameter 5)*

517 The cinnamyl/G ratio, generally used to differentiate between non-woody and woody
518 tissue (Hedges and Mann, 1979), may be adequate for anaerobic systems with large
519 changes in vegetation type (e.g. Tareq et al., 2006). However, no such correlation was
520 found for the Penido Vello peat (not shown), probably because most G lignin pyrolysis
521 products were as similarly rapidly degraded as 4-vinylguaiacol (from ferulic acid).
522 However, the ratio 4-vinylguaiacol to lignin and the ratio of 4-vinylphenol (from *p*-
523 coumaric acid) to H moieties are good indicators for graminoids, particularly those that
524 perform better under relatively wet conditions (*Eriophorum* and *Molinia*, Section 3.2.4),
525 which is in agreement with their abundance in peatland plants (Schellekens et al., 2012).
526 The association of parameter 5a with graminoids is possibly caused by the fact that H
527 moieties were related to ericoids (Section 3.2.2.2).
528
529 *3.3.6. Hemicellulose and lignified cellulose (parameters 6 and 7)*
530 During the first stage of decay hemicellulose and cellulose are degraded at equal
531 rates, but relatively slowly compared with other polysaccharides. During long term
532 anaerobic decay, hemicellulose is preferentially lost over cellulose; this was also found
533 for *Calluna* tissue in peat and is presumably related to protection of cellulose by lignin
534 (van der Heijden and Boon, 1994), in agreement with the high abundance of cellulose
535 derived pyrolysis products found in residue peat samples by Schellekens et al. (2012).
536
537 *3.4. C/N ratio*
538 Carbon content (corrected for ash, r^2 0.75) and C/N (r^2 0.52) were positively
539 correlated with F1, while nitrogen content was negatively correlated (corrected for ash,
540 r^2 0.50; Fig. 3). These data indicate that higher C/N values were found in more
541 decomposed samples (Pontevedra-Pombal et al., 2004). This contradicts the frequent

542 use of C/N to reflect decomposition in *Sphagnum*-dominated peat (Malmer and Holm,
543 1984; Kuhry and Vitt, 1996). Although high C/N, after detrending from the long-term
544 depth trend, corresponded to wet periods indicated by non-pollen palynomorphs in a
545 nearby peatland with similar vegetation type (Martínez-Cortizas et al., 2007), the huge
546 difference in variability of the C/N record in *Sphagnum* peatlands (high variability; e.g.
547 Schellekens and Buurman, 2011) compared to graminoid-dominated peatlands (low
548 variability), suggests that the variation of C/N is strongly influenced by vegetation type
549 as pyrolysis parameters showed high variability in both vegetation types. The positive
550 correlation of carbon with F1 presumably reflects the loss of polysaccharides during
551 initial decay (Schellekens, 2013). High values of nitrogen in the upper part of the core
552 were mainly responsible for the negative correlation of nitrogen and F1 (Fig. 3),
553 probably reflecting the rapid recycling of nitrogen by plants in the aerobic layer.
554

555 **4. Conclusions**

556 Factor analysis applied to pyrolysates of bulk peat sampled at high resolution
557 allowed to distinguish effects of environmental factors on the Penido Vello peat
558 chemistry. Identified environmental factors included surface decay (both anaerobic and
559 aerobic), depth related decay (anaerobic), vegetation shifts (graminoids vs. ericoids) and
560 sub-surface decay (aerobic), with decreasing impact on peat chemistry.

561 Decay at the surface showed greatest influence on peat chemistry, and is best
562 reflected in the lignin/polysaccharide and S/G ratios, indicating that the major change in
563 peat chemistry at the surface is a loss of easily degradable polysaccharides and non-
564 lignin phenolic guaiacyl moieties under aerobic conditions. Depth related anaerobic
565 decay showed minor effects on most lignin parameters, and was only significant for
566 polysaccharide related parameters, indicating a decrease of hemicellulose and an

567 increase of cellulose with depth, the latter reflecting lignified cellulose. Although
568 secondary aerobic decay in the sub-surface was less prominent in the overall peat
569 chemistry, for the lignin parameters it showed a higher impact than depth related
570 anaerobic decay. Sub-surface decay resulted in a loss of S and G moieties with a C_3
571 alkyl side chain, while moieties with acetyl side chains increased. The allocation of the
572 effects of different processes on lignin parameters in combination with depth records of
573 high-resolution sampled peat allowed to determine the net effect on bulk peat, and their
574 usefulness as decomposition proxies.

575 The lignin composition of the studied peat core was predominantly determined by
576 decay. However, the dominance of graminoids was the main factor that determined the
577 consequences of decay on the composition of lignin phenols and considerably differed
578 from that of woody tissue. Our results support the idea that lignin decay is a surface
579 process, and that anatomical structure or accessibility control the decay of lignocellulose
580 at different scales (van der Heijden and Boon, 1994; Grabber et al., 1997, 2004; Love et
581 al., 1998; Talbot et al., 2012). Because plant groups differ in chemistry a general decay
582 mechanism may not be valid for peat composed of unknown mixtures of different plant
583 groups (ericoids-graminoids-mosses). For the reconstruction of peat water surface
584 wetness from lignin chemistry we recommend the use of a number of vegetation and
585 decomposition proxies that provide similar information.

586

587 **Acknowledgements**

588 This work was partially supported by projects CGL2010-20672 (Spanish Ministerio de
589 Ciencia e Innovación) and 10PXIB200182PR (Dirección Xeral de I+D, Xunta de
590 Galicia). The ^{13}C CPMAS NMR analysis by the fifth author was supported by the
591 European Community Activity Large-Scale Facility Wageningen NMR Centre (grant

592 ERBFMGECT950066). We thank B. van Lagen and E. Velthorst of Wageningen
593 University for preparation of pyrograms and two anonymous reviewers for constructive
594 comments.
595

596 **References**

597 Abbott, G.D., Swain, E.Y., Muhammad, A.B, Allton, K., Belyea, L.R., Laing, C.G.,
598 Cowie, G.L., 2013. Effect of water-table fluctuations on the degradation of
599 *Sphagnum* phenols in surficial peats. Geochimica et Cosmochimica Acta 106, 177-
600 191.
601 Béguin, P., Aubert, J.P., 1994. The biological degradation of cellulose. FEMS
602 Microbiology Reviews 13, 25–58.
603 Benner, R., MacCubbin, A.E., Hodson, R.E., 1984. Anaerobic biodegradation of the
604 lignin and polysaccharide components of lignocellulose and synthetic lignin by
605 sediment microflora. Applied and Environmental Microbiology 47, 998–1004.
606 Blackford, J.J., Chambers, F.M., 1993. Determining the degree of peat decomposition
607 for peat-based palaeoclimatic studies. International Peat Journal 5, 7–24.
608 Boon, J.J., Wetzel, R.G., Godshalk, G.L., 1982. Pyrolysis mass spectrometry of some
609 Scirpus species and their decomposition products. Limnology and Oceanography 27,
610 839–848.
611 Bourdon, S., Laggoun-Défarge, F., Disnar, J., Maman, O., Guillet, B., Derenne, S.,
612 Largeau, C., 2000. Organic matter sources and early diagenetic degradation in a
613 tropical peaty marsh (Tritrivakely, Madagascar). Implications for environmental
614 reconstruction during the Sub-Atlantic. Organic Geochemistry 31, 421–438.

615 Broder, T., Blodau, C., Biester, H., Knorr, K.H., 2012. Peat decomposition records in
616 three pristine ombrotrophic bogs in southern Patagonia. Biogeosciences 9, 1479–
617 1491.
618 Buranov, A.U., Mazza, G., 2008. Lignin in straw of herbaceous crops. Industrial Crops
619 and Products 28, 237–259.
620 Buurman, P., Nierop, K.G.J., Pontevedra-Pombal, X., Martínez-Cortizas, A., 2006.
621 Molecular chemistry by pyrolysis–GC/MS of selected samples of the Penido Vello
622 peat deposit, NW Spain, in: Martini, I.P., Martínez-Cortizas, A., Chesworth, W.
623 (Eds.), Peatlands. Evolution and records of environmental and climate changes.
624 Elsevier, Amsterdam, pp. 217–240.
625 Chen, L., Auh, C., Chen, F., Cheng, X., Aljoe, H., Dixon, R.A., Wang, Z., 2002. Lignin
626 deposition and associated changes in anatomy, enzyme activity, gene expression, and
627 ruminal degradability in stems of tell fescue at different development stages. Journal
628 of Agricultural Food Chemistry 50, 5558–5565.
629 Christman, R.F., Oglesby, R.T., 1971. Microbial degradation and the formation of
630 humus. In: Sarkanen, K.V., Ludwig, C.H., (Eds), Lignins. Wiley, NewYork, pp.
631 769–795.
632 Clymo, R.S., Turunen, J., Tolonen, K., 1998. Carbon accumulation in peatland. Oikos
633 81, 368–388.
634 del Rio, J.C., Gutiérrez, A., Rodríguez, I.M., Ibarra, d., Martínez, A.T., 2007.
635 Composition of non-woody plant lignins and cinnamic acids by Py-GC/MS,
636 Py/TMAH and FTIR. Journal of Analytical and Applied Pyrolysis 79, 39–46.
637 Disnar, J.R., Jacob, J., Morched-Issa, M., Lottier, N., Arnaud, F., 2008. Assessment of
638 peat quality by molecular and bulk geochemical analysis: Application to the

639 Holocene record of the Chautagne marsh (Haute Savoie, France). Chemical Geology
640 254, 101–112.
641 Donaldson, L.A., 2001. Lignification and lignin topochemistry – an ultrastructural view.
642 Phytochemistry 57, 859–873.
643 Eglinton, G., Hamilton, R.J., 1967. Leaf epicuticular waxes. Science 156, 1322–1325.
644 Ertel, J.R., Hedges, J.I., 1985. Sources and sedimentary humic substances: vascular
645 plant debris. Geochimica et Cosmochimica Acta 49, 2097–2107.
646 Fraga, I., Romero-Pedreira, D., Sahuquillo, E., 2005. Patterns of vegetation in relation
647 to environmental conditions in Northwestern Spanish mires. IMCG Mires and
648 Peatlands Field Symposium, Tierra del Fuego 2005, Argentina, pp. 35–36.
649 Filley, T.R., Cody, G.D., Goodell, B., Jellison, J., Noser, C., Ostrofsky, A., 2002.
650 Lignin demethylation and polysaccharide decomposition in spruce sapwood
651 degraded by brown rot fungi. Organic Geochemistry 33, 111–124.
652 Filley, R.T., 2003. Assessment of fungal wood decay by lignin analysis using
653 tetramethylammonium hydroxide (TMAH) and ^{13}C labeled TMAH
654 thermochemolysis, in: Goodell, B., Nicholas, D.D., Schultz T.P. (Eds.), Wood
655 deterioration and preservation - Advances in our changing world. ACS
656 SYMPOSIUM SERIES 845, pp. 119–139.
657 Geissman, T.A., Crout, D.H.G., 1969. Organic chemistry of secondary plant
658 metabolism. Freeman, Cooper and Co., San Francisco.
659 Grabber, J.H., Ralph, J., Hatfield, R.D., Quideau, S., 1997. *p*-Hydroxyphenyl, guaiacyl,
660 and syringyl lignins have similar inhibitory effects on wall degradability. Journal of
661 Agricultural and Food Chemistry 45, 2530–2532.

662 Grabber, J.H., Ralph, J., Lapierre, C., Barriére, Y., 2004. Genetic and molecular basis of
663 grass cell-wall degradability. I. Lignin-cell wall matrix interactions. Comptes Rendus
664 Biologies 327, 455–465.
665 Harman-Ware, A.E., Crocker, M., Kaur, A.K., Meier, M.S., Kato, D., Lynn, B., 2013.
666 Pyrolysis-GC/MS of synapyl and coniferyl alcohol. Journal of Analytical and
667 Applied Pyrolysis 99, 161–169.
668 Healy, J.B., Young, L.Y., Reinhard, M., 1980. Methanogenic decomposition of ferulic
669 acid, a model lignin derivative. Applied Environmental Microbiology 39, 1137–
670 1152.
671 Hedges, J.I., Mann, D.C., 1979. The characterization of plant tissues by their lignin
672 oxidation products. Geochimica et Cosmochimica Acta 43, 1803–1807.
673 Hedges, J.I., Blanchette, R.A., Weliky, K., Devol, A.H., 1988. Effects of fungal
674 degradation on the CuO oxidation products of lignin: a controlled laboratory study.
675 Geochimica et Cosmochimica Acta 52, 2717–2726.
676 Higuchi, 1990. Lignin biochemistry: biosynthesis and biodegradation. Wood Science
677 and Technology 24, 23–63.
678 Huang, Y., Stankiewicz, B.A., Eglinton, G., Snape, C.E., Evans, B., Latter, P.M.,
679 Ineson, P., 1997. Monitoring biomacromolecular degradation of *Calluna vulgaris* in
680 a 23 year field experiment using solid state ^{13}C-NMR and pyrolysis-GC/MS. Soil
681 Biology & Biochemistry 30, 1517–1528.
682 Jex, C.N., Pate, G.H, Blyth, A.J., Spencer, R.G.M., Hernes, P.J., Khan, S.J., Baker, A.,
683 2014. Lignin biogeochemistry: from modern processes to Quaternary archives.
684 Quaternary Science Reviews 87, 46–59.
685 Jung, H.J.G., Casler, M.D., 1990. Lignin concentration and composition of divergent
686 smooth bromegrass genotypes. Crop Science 30, 980–985.

687 Kaal, J., Baldock, J.A., Buurman, P., Nierop, K.G.J., Pontevedra-Pombal, X., Martínez-
688 Cortizas, A., 2007. Evaluating pyrolysis-GC/MS and ^{13}C CPMAS NMR in
689 conjuction with a molecular mixing model of the Penido Vello peat deposit, NW
690 Spain. Organic Geochemistry 38, 1097–1111.
691 Kirk, T.K., Farrel, R.L., 1987. Enzymatic combustions – the microbial degradation of
692 lignin. Annual Review of Microbiology 41, 465–505.
693 Kögel, I., 1986. Estimation and decomposition pattern of the lignin component in forest
694 humus layers. Soil Biology & Biochemistry 18, 589–594.
695 Kögel-Knabner, I., 2002. The macromolecular organic composition of plant and
696 microbial residues as inputs to soil organic matter. Soil Biology & Biochemistry 34,
697 139–162.
698 Kondo, T., Mizuno, K., Kato, T., 1989. Cell wall-bound *p*-coumaric and ferulic acids in
699 Italian ryegrass. Canadian Journal of Botany 71, 495–499.
700 Kuder, T., Kruge, M.A., 1998. Preservation of biomolecules in sub-fossil plants from
701 raised peat bogs – a potential paleoenvironmental proxy. Organic Geochemistry 29,
702 1355–1368.
703 Kuder, T., Kruge, M.A., Shearer, J.C., Miller, S.L., 1998. Environmental and botanical
704 controls on peatification – a comparative study of two New Zealand restiad bogs
705 using Py-GC/MS, petrography and fungal analysis. International Journal of Coal
706 Geology 37, 3–27.
707 Kuhry, P., Vitt, D.H., 1996. Fossil carbon/nitrogen as a measure of peat decomposition.
708 Ecology 77, 271–317.
709 Kuroda, K., 2000. Analytical pyrolysis products derived from cinnamyl alcohol-end
710 groups in lignins. Journal of Analytical and Applied Pyrolysis 53, 123–134.

711 Lam, T.B.T., Iiyama, K., Stone, B.A., 1992. Changes in phenolic acids from internode
712 walls of wheat and phalaris during maturation. Phytochemistry 31, 2655–2658.
713 Love, G.D., Snape, C.E., Jarvis, M.C., 1998. Comparison of leaf and stem cell-wall
714 componentes in barley straw by solid-state ^{13}C NMR. Phytochemistry 49, 1191–
715 1194.
716 Machinet, G.E., Bertrand, I., Barrière, Y., Chabbert, B., Recous, S., 2011. Impact of
717 plant cell wall network on biodegradation in soil: role of lignin composition and
718 phenolic acids in roots from 16 maize genotypes. Soil Biology & Biochemistry 43,
719 1544–1552.
720 Malmer, N., Holm, E., 1984. Variation in the C/N quotient of peat in relation to
721 decomposition rate and age determination with ^{210}Pb. Oikos, 43, 171–182.
722 Malmer, N., Svensson, B.M., Wallén, B., 1994. Interactions between *Sphagnum* mosses
723 and field layer vascular plants in the development of peat-forming systems. Folia
724 Geobotanica et Phytotaxonomica 29, 483–496.
725 Martínez-Cortizas, A., Pontevedra-Pombal, X., Nóvoa-Muñoz, J. C., García-Rodeja, E.,
726 1997. Four thousand years of atmospheric Pb, Cd and Zn deposition recorded by the
727 ombrotrophic peat bog of Penido Vello (Northwestern Spain). Water Air and Soil
728 Pollution 100, 387–403.
729 Martínez-Cortizas, A., García-Rodeja, E., Pontevedra-Pombal, X., Nóvoa-Muñoz, J.C.,
730 Weiss, D., Cheburkin, A., 2002. Atmospheric Pb deposition in Spain during the last
731 4600 years recorded by two ombrotrophic peat bogs and implications for the use of
732 peat as archive. Science of the Total Environment 292, 33–44.
733 Martínez-Cortizas, A., Biester, H., Mighall, T., Bindler, R., 2007. Climate-driven
734 enrichments of pollutants in peatlands. Biogeosciences 4, 905–911.

735 Mason, S.L., Filley, T.R., Abbott, G.D., 2009. The effect of afforestation on the soil
736 organic carbon (SOC) of a peay gleysol using on-line thermally assisted hydrolysis
737 and methylation (THM) in the presence of ^{13}C-labelled tetramethylammonium
738 hydroxide (TMAH). Journal of Analytical and Applied Pyrolysis 85, 417–425.
739 Meier, D., Faix, O., In: Lin, S.Y., Dence, C.W. (Eds.), Methods in lignin chemistry,
740 1992, pp. 177.
741 Mulder, M.M., Pureveen, J.B.M., Boon, J.J., Martinez, A.T., 1991. An analytical
742 pyrolysis mass spectrometric study of *Eucryphia coridifolia* wood decayed by white-
743 rot and brown-rot fungi. Journal of Analytical and Applied Pyrolysis 19, 175–191.
744 Nierop, K.G.J., Filley, T.R., 2007. Assessment of lignin and (poly)phenol
745 transformations in oak (*Quercus robur*) dominated soils by 13C-TMAH
746 thermochemolysis. Organic Geochemistry 38, 551–565.
747 Opsahl, S., Benner, R., 1993. Decomposition of senescent blades of the seagrass
748 *Halodule wrightii* in a subtropical lagoon. Marine Ecology Progress Series 94, 191–
749 205.
750 Opsahl, S., Benner, R., 1995. Early diagenesis of vascular plant tissues: lignin and cutin
751 decomposition and biogeochemical implications. Geochimica et Cosmochimica Acta
752 59, 4889–4904.
753 Philben, M., Kaiser, K., Benner, R., 2013. Does oxygen exposure time control the
754 extent of organic matter decomposition in peatlands? Journal of Geophysical
755 Research: Biogeosciences 119, 897–909.
756 Pontevedra-Pombal, X., Martínez-Cortizas, A., García-Rodeja, E., Buurman, P., van
757 Lagen, B., 2001. Composición y transformación de la materia orgánica en un histosol
758 de la serra do Xistral (norte de Galicia) mediante CPMAS ^{13}C-NMR. Edafologia 8,
759 67–79.

760 Pontevedra-Pombal, X., Martínez-Cortizas, A., Buurman, P., 2004. Las turberas de
761 montaña de Galicia como sumideros de carbono. Edafologia 11, 295–307.
762 Pouwels, A.D., Tom, A., Eijkel, G.B., Boon, J.J., 1987. Characterisation of beech wood
763 and its holocellulose and xylan fractions by pyrolysis-gas chromatography-mass
764 spectrometry. Journal of Analytical and Applied Pyrolysis 11, 417–436.
765 Pouwels, A.D., Eijkel, G.B., Boon, J.J., 1989. Curie-point pyrolysis high-resolution gas
766 chromatography–mass spectrometry of microcrystalline cellulose. Journal of
767 Analytical and Applied Pyrolysis 14, 237–280.
768 Ralph, J., Hatfield, R.D., 1991. Pyrolysis-GC–MS characterization of forage materials.
769 Journal of Agricultural and Food Chemistry 39, 1426–1437.
770 Saiz-Jimenez, C., de Leeuw, J.W., 1984. Pyrolysis-gas chromatography-mass
771 spectrometry of isolated, synthetic and degraded lignins. Organic Geochemistry 6,
772 417–422.
773 Schellekens, J., Buurman, P., Pontevedra-Pombal, X., 2009. Selecting parameters for
774 the environmental interpretation of peat molecular chemistry – a pyrolysis-GC/MS
775 study. Organic Geochemistry 40, 678–691.
776 Schellekens, J., Buurman, P., 2011. *n*-Alkane distributions as palaeoclimatic proxies in
777 ombrotrophic peat: the role of decomposition and dominant vegetation. Geoderma
778 164, 112–121.
779 Schellekens, J., Buurman, P., Fraga, I., Martínez-Cortizas, A., 2011. Holocene
780 hydrologic changes inferred from molecular vegetation markers in peat, Penido Vello
781 (Galicia, Spain). Palaeogeography, Palaeoclimatology, Palaeoecology 299, 56–69.
782 Schellekens, J., Buurman, P., Kuyper, T.W., 2012. Source and transformations of lignin
783 in *Carex*-dominated peat. Soil Biology & Biochemistry 53, 32–42.

784 Schellekens, J., 2013. The use of molecular chemistry (pyrolysis-GC/MS) in the
785 environmental interpretation of peat. Ph.D. thesis, Wageningen University.
786 Sinsabaugh, R.L., 2010. Phenol oxidase, peroxidase and organic matter dynamics of
787 soil. Soil Biology & Biochemistry 42, 391–404.
788 Smith, B.G., Harris, P.J., 1999. The polysaccharide composition of Poales cell walls:
789 Poaceae cell walls are not unique. Biochemical Systematics and Ecology 27, 33–53.
790 Sun, X.F., Jing, Z., Fawler, P., Wu, T., Rajaratnam, M., 2011. Structural
791 characterization and isolation of lignin and hemicelluloses from barley straw.
792 Industrial Crops and Products 33, 588–598.
793 Talbot, J.M., Yelle, D.J., Nowick, J., Treseder, K.K., 2012. Litter decay rates are
794 determined by lignin chemistry. Biogeochemistry 108, 279–295.
795 Tareq, S.M., Handa, N., Tanoue, E., 2006. A lignin phenol proxy record of mid
796 Holocene paleovegetation changes at Lake DaBuSu, northeast China. Journal of
797 Geochemical Exploration 88, 445–449.
798 Thevenot M., Dignac, M.F., Rumpel, C., 2010. Fate of lignins in soils: a review. Soil
799 Biology & Biochemistry 42, 1200–1211.
800 Tsutsuki, K., Esaki, I., Kuwatsuka, S., 1994. CuO-oxidation products of peat as a key to
801 the analysis of the paleo-environmental changes in a wetland. Soil Science and Plant
802 Nutrition 40, 107–116.
803 van der Hage, E.R.E., Mulder, M.M., Boon, J.J., 1993. Structural characterization of
804 lignin polymers by temperature-resolved in-source pyrolysis-mass spectrometry and
805 Curie-point pyrolysis-gas chromatography/mass spectrometry. Journal of Analytical
806 and Applied Pyrolysis 25, 149–183.

van der Heijden, E., Boon, J.J., 1994. A combined pyrolysis mass spectrometric and light microscopic study of peatified *Calluna* wood isolated from raised bog peat deposits. Organic Geochemistry 22, 903–919.

van der Heijden, E., Boon, J.J., Bouman, F., 1994. Anatomy of native and peatified *Calluna vulgaris* stems: implications for coal maceral formation. International Journal of Coal Geology 25, 1–25.

Wende, G., Fry, S.T., 1996. 2-*O*-β-D-xylopyranosyl-(5-*O*-Feruloyl)-L-arabinose, a widespread component of grass cell walls. Phytochemistry 44, 1019–1030.

Williams, C.J., Yavitt, J.B., 2003. Botanical composition of peat and degree of peat decomposition in three temperate peatlands. Ecoscience 10, 85–95.

Wilson, J.O., Valiela, I., Swain, T., 1985. Sources and concentrations of vascular plant material in sediments of Buzzards Bay, Massachusetts, USA. Marine Biology 90, 129–137.

Yeloff, D., Mauquoy, D., 2006. The influence of vegetation composition on peat humification: implications for palaeoclimatic studies. Boreas 35, 662–673.

Young, L.Y., Frazer, A.C., 1987. The fate of lignin and lignin-derived compounds in anaerobic environments. Geomicrobiology Journal 5, 261–293.

Zaccone, C., Said-Pullicino, D., Gigliotti, G., Miano, T.M., 2008. Diagenetic trends in the phenolic constituents of *Sphagnum*-dominated peat and its corresponding humic acid fraction. Organic Geochemistry 39, 830–838.